THE ŒNOCYTES OF PLATYPHYLAX DESIGNATUS WALKER.

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INTRODUCTION.

The œnocytes as unique cells of the body of all of the orders of insects which have been investigated, except the Aptera, have received much attention within the last couple of decades, but with relatively little more than mere conjecture as to their definite function. With the idea of throwing added light on the nature of the activities of these cells, the investigations here discussed were made on the larvæ, pupæ, and adults of *Platyphylax designatus*, a caddis-fly, which occurs quite abundantly in fresh-water springs in the vicinity of Madison, Wis.

The results here presented were derived from a study of the structure of the œnocytes in several periods of the life-history of this insect, and by injection experiments. Several stains were tried, Heidenhain s iron-hæmatoxylin proving the best for detailed work. Delafield's hæmatoxylin with orange G or eosin were the stains used in determining the location and number of the œnocytes. The cytoplasm of the œnocytes takes up eosin very readily, as it does also orange G. The substances used in injections were methylene blue as an intra-vitam stain and sulphindigotate of sodium to test the excretory nature of the cells.

The papers of Perez (1903), Rössig (1904), and Weissenberg (1907), presenting a rather complete review of the work done on œnocytes, it will here be necessary only to briefly mention some of the chief contributors to the subject.

According to Rössig (1904), the first to observe the œnocytes as special organs was M. Fabre (1856).

Landois (1865) distinguished them from the fat-body, and gave to them the name "Respirationszellen" because of the relation he observed them to bear to the tracheæ.

Haberlandt (1872) observed them in the silk-worm, where they were described by him as an organ of unknown function.

Graber (1873) separated the œnocytes sharply from the pericardial cells, and called them "eingesprengte Zellen." He said regarding their function: "Ohne Zweifel haben wir es hier mit eigenartigen einzelligen Drüsen zu thun, über deren secret allerdings nicht das Geringste bekannt ist."

The name "Oenocythen" was given to these cells by Wielowiejski (1886) on account of their resemblance in color, in the larva of *Chironomus*, to white wine. They were observed by him to be metamerically repeated under the hypodermis of the abdominal segments and arranged in small groups surrounding or near to the stigmata. This was the most extensive work on the œnocytes up to this time; for Wielowiejski was successful in identifying them in the Diptera, Coleoptera, Hymenoptera and Lepidoptera. He includes the œnocytes among the elements of the blood tissue and says regarding these: ". . . dass sie alle von dem sie umgebenden Medium gewisse Stoffe aufnehmen, zeitweise aufspeichern resp. verarbeiten und irgend welche Umsatzprodukte an dasselbe zurückgeben und dadurch auf die in den Hauptgeweben des Organismus vor sich gehenden Assimilations- und Desassimilationsprocesse einen Einfluss ausüben."

Kowalevsky (1887) attributed to the œnocytes a glandular function.

Verson (1891–2) in his work on the silk-worm gives them the name "cellule glandulari hypostigmatische," and finds their location exclusively under and behind the stigmata of the abdominal segments. As evidenced by the number of vacuoles in their cytoplasm during the larval stages, he attributes to them a glandular function. At the time of the spinning of the cocoon, two or three days before pupation, a second œnocyte generation appears in broad layers on the ventral part of the third, fourth, and the fifth abdominal segments. These attain a size of 60μ in the six-day pupa, and begin at this time to divide by amitosis. The larval œnocytes persist to the end of the life of the individual, reaching as much as 136μ in size.

Graber (1891) places the œnocytes with the blood tissue and gives their origin as ectodermal. He, however, considers them

as being metamorphosed into the fat-body, and also as giving rise to the blood corpuscles.

Wheeler (1892) in an extensive paper on the blood tissue of insects deals with the œnocytes at considerable length. He arrives at the following conclusions:

1. "The œnocytes are derived by delamination from the ectoderm just caudad to the tracheal involutions. They are also metameric organs.

2. "They are limited to the eight trachigerous abdominal segments.

3. "They appear to be restricted to the Pterygota, in all the members of which group they probably occur.

4. "They give rise neither to the fat-body nor to the blood but represent organs *sui generis*.

5. "After their differentiation from the primitive ectoderm, they never divide, but gradually increase in size."

With regard to their function, the only suggestion that Wheeler makes is in his discussion of their vacuolate structure in certain of the larvæ of the Trichoptera. Here he says: "One is reminded of certain gland cells which store up vacuoles of a specific substance in their cytoplasm, preparatory to secretion."

Heymons (1895) in the embryogenesis of *Forficula*, points out that the œnocytes are found in the eleventh abdominal segment as well as in the others. The eleventh segment bears no stigma, and thus they cannot bear any relation to the stigma as such.

Pantel (1898) classes the œnocytes as an organ because of their metameric repetition, and treats to a slight extent of their cytological changes in different periods of the insect's life. He hesitates, however, to commit himself as to their function, but rather inclines toward the excretory phases of their probable activities.

Berlese (1899), in his work on the ants, *Tapinoma erraticum* and *Pheidole pallidula*, finds that, in the beginning of metamorphosis, the larval œnocytes are multiplied, give up their fixed position, and, becoming amœboid, are scattered about among the fat-bodies. In his work on *Polistes gallica* and on the honey-bee, he finds a persistence of the œnocytes, with no special second generation. Their function he considers as excretory,

serving during the periods of moulting and pupation, when the Malpighian tubes are functionless.

Anglas (1900) describes the œnocytes as more or less amœboid, and with no special distribution in the Hymenoptera, except their existence in the abdomen alone. Except for a relative increase in volume, they remain throughout the life-cycle, in both distribution and structure, very similar to what they are in the early larval stages. He suggests that they perhaps secrete about themselves ferments, and considers them as glandular cells originating from the hypodermis and functioning as organs of internal secretion perhaps for dissociative purposes. The products of their secretion may serve for the general nutrition of the tissues of the body or even for the cytolysis of those larval cells destined to disappear.

Koschevnikov (1900), in his work on the fat-body and œnocytes of the honey-bee, finds that most of the large œnocytes of the larva disintegrate in the pupal stage; some of them continuing, however, into the beginning of the imaginal stage. In a young pupa, a second œnocyte generation, to which he gives the name "Imaginalœnocyten," arises from the hypodermis, and these œnocytes are later distributed among the fat-bodies. These are only one fifth as large as the larval œnocytes and are formed only in the pupal stage. He observed inclusions in some of the cells, and on this basis came to the conclusion that they are to be considered as excretory organs, without "Ausführungsgange," which serve as storage places for excess products of excretion.

Vaney (1902) says: "Chez la *Simulia*, le *Culex*, le *Chironomus*, durant la période nymphale je ne vois aucun changement dans ces cellules." However, he observes in *Simulium* that the number of œnocytes appears to have increased at the end of pupation. He concludes: "Chez les *Dipteres* les œnocytes ne subissent aucune histolyse durant la nymphose."

Perez (1903) states relative to the discovery of Koschevnikov, concerning the origin of a second œnocyte generation, that this opinion seems to be erroneous.

Rössig (1904) finds that, in the Cynipidæ, the œnocytes are limited to the abdomen. The larval œnocytes reach a length as great as 150μ and attain a proportionate length one fifth that

of the body. These larval œnocytes degenerate in the pupal stage. The imaginal œnocytes originate at about the same time through mitotic division of the hypodermal cells on the ventral side of the abdominal segments. They are described as very much smaller than the larval œnocytes, never equalling them in size.

Weissenberg (1907) finds in a well grown larva of *Torymus nigricornis*, over one hundred œnocytes, presenting no definite arrangement, and scattered about among the fat-bodies. They are in no way limited to the abdomen, nor do they necessarily have the definite tracheal attachments observed by Wielowiejski and others. The principal degeneration of the larval œnocytes occurs in the stage which he calls the "gelben Puppe." The larvæ shortly before pupation contain the imaginal œnocytes in the form of special œnocyte imaginal plates, which are laid in the niches of the dorsal hypodermis in the fifth to eleventh abdominal segments.

With regard to the function of these cells he says: "Schon oben habe i h mich gegen die Auffassung der Zelleinschlüsse als Exc ete ausgesprochen, möchte aber hier nicht das für Urate characterische Bild der braunen, radiär gestreiften, runden Krystallkörper darbieten, sondern an den frischen Zellen homogen und farblos erscheinen. '

Original Observations.

In the larvæ of *Platyphylax designatus*, the œnocytes are limited to the abdomen. A careful search through serial sections of the thorax of several individuals in different larval stages showed no traces of œnocytes in this portion of the body. They are in no way limited, however, to certain abdominal segments but occur in all, the larger number being present in segments two to seven. The definite tracheal attachments observed by many of the workers on this subject have not been seen in this insect. This observation agrees essentially with that of Weissenberg (1907) in *Torymus nigricornis:* "Von einer Befestigung an Tracheencapillaren, habe ich nichts bemerkt."

The œnocytes occur both dorsally and ventrally, by far the greater number occupying the latter position. The larger œno-

cytes, so conspicuous in the early larval stages, occur ventrally in pairs, one on each side of every abdominal segment. The smaller œnocytes of the earlier, as well as the later larval stages, are found principally in groups of from two to five, the larger groups appearing more ventrad. In several specimens they were observed near to or even grouped around a tracheal tube to which they, however had no connection. The location of all of the œnocytes is distinctly pleural.

The distribution of the œnocytes does not change essentially with the increase in size of the larvæ, but there is a less marked tendency for them to occur in groups. With the increase in size of the fat-body more of them become partially or completely surrounded by this structure. These changes are more marked in the late larval and in the pupal stages, but we frequently find in these periods, groups of as many as five œnocytes, and this tendency to grouping is observed even in the imaginal stage.

A careful count of the œnocytes of a larva eight millimeters in length totals about one hundred and twenty-five, these being approximately equally proportioned between the right and left sides of the body. From other estimates it is found that this number is about typical for *Platyphylax designatus*. There seems to be no wide fluctuation in numbers in different periods of the life-history, though the imaginal stages appear to show a decrease in number over the larval, due perhaps to disintegration. The conclusion that the number is constant for all the larvæ of the same stage, is, I believe, hardly justifiable, but there is no wide variation in this regard. In no case, have I seen evidences of division either by mitosis or amitosis in any of the stages studied, nor an increase in number in later larval, pupal or imaginal stages over that in the earlier larval periods.

While the œnocytes appear practically constant in number throughout the stages examined, there is to be noted a very marked increase in their size as the larva advances in growth. The following table represents the size of an average œnocyte derived from measurements of several of these cells in the stages indicated.

The largest size of the œnocytes is reached in the pupal stage; the greatest length of the nucleus proportionate to that of the

Length of Larva.	Length of Cell.	Length of Nucleus.
4.5 mm.	18.4μ	10.4μ
6 mm.	24 μ	12 μ
12 mm.	40 μ	20.6μ
22 mm.	77 μ	43.5μ
pupa	103.1μ	63.5μ
early imago	94.3μ	62.9μ

cell is found in the imaginal stage. There is to be noted, however, a decrease in the total length of the œnocytes of this latter stage, this being due, beyond doubt, to degeneration, manifest signs of which are present.

There are two sizes of œnocytes in the earlier larval stages. One of the larger type, from a larva six millimeters in length, is shown in the accompanying plate (Fig. 3). As already noted, these large œnocytes are present in each of the abdominal segments, ventral in position, one occurring on each side. These are many times larger than the œnocytes of the smaller type, and are pressed rather closely against the hypodermis. In the earlier stages of larval life, the greater comparative size makes these œnocytes very conspicuous, while in the older larva this is not so marked; in fact the smaller œnocytes in the oldest larvæ attain a size equal to or even greater than the formerly larger ones. Careful examination of serial sections of these larger œnocytes showed no traces of a duct leading from them.

The smaller œnocytes are more numerous than the larger. In the early larval stages, they are, as noted above, much smaller than the other type, attaining, in the old larvæ, an equal or greater size. Their distribution in the body is, however, very much more irregular.

In the earlier larval periods the œnocytes appear much more rounded in form than in the earlier stages. The nucleus shows the same tendency. The cytoplasm presents a finely granular, homogeneous appearance, and takes up rather evenly the orange G and eosin. In these stages, however, the cytoplasm appears rather more dense than in the succeeding periods.

Wheeler says with regard to the œnocytes of an unidentified phryganeid larva on which he worked that the nuclei contain no *nucleole*. In a 4.5 mm. stage of *Platyphylax designatus*, stained with safranin, a nucleole is seen to stand out very clearly (Fig. 1).

This apparently disappears in later stages. The bulk of the chromatin appears in small, rounded granules connected to form a skein. One easily observes several larger masses of chromatin standing out rather conspicuously in the nuclei of the œnocytes of all the larval stages. The nuclear membrane appears sharply defined in all of the stages except the imaginal.

Besides a relative increase in the size of the cell, there appears little change in the character of the œnocytes up to the last larval stage. At this period the nucleus becomes proportionately much larger and more irregular in outline. The cytoplasm shows, between the nuclear membrane and cell boundary, an inner and an outer zone or layer. The more central of these two layers shows the cytoplasm to contain more and larger vacuoles, and to have a denser appearance; the outer, smaller zone, on the contrary, contains fewer and smaller vacuoles. None of these vacuoles are clear, but one may distinguish in the larger ones, by careful focusing, many very small granules, which would seem to indicate the presence of more than a homogeneous content. These vacuoles have been observed by Verson (1891–2), Wheeler (1892), and others, all of whom interpret them as indicative of secretory activity. Wheeler (1892) says: "These vacuoles are but slightly refractive, and are not fat globules. This condition of the œnocytes was found in a number of larvæ, and I believe, represents a normal physiological state. One is reminded of certain gland cells which store up vacuoles of a specific substance in their cytoplasm, preparatory to secretion."

In the pupal stages, one finds that the nucleus has a more irregular outline, and that the cytoplasm contains comparatively few vacuoles. It is in this stage that the œnocytes attain their largest growth. This fact coupled with the appearances of activity in the late larval stages may perhaps be taken as indications of a greater secretory activity of these cells during these two periods.

In the imago, just after emergence, the œnocytes show marked signs of degeneration. The boundary of the cells has become indistinct and frayed, and the outer portion of the cytoplasm is stained rather deeply with the hæmatoxylin (Fig. 7). At this stage, the nuclear membrane is not distinguishable, and but few

vacuoles are to be noted in the cytoplasm. The chromatin has gathered into irregular masses along the sides of the adjacent cytoplasm (position of former nuclear membrane) and the linin appears in masses which have separated from the chromatin material. In an imago two or three days after emergence, but which had been kept enclosed in a small dish, the degeneration of the nucleus is seen to have proceeded further. The cell does not, however, present an essentially different appearance to that shown in the imago two or three hours after emergence. This third-day imago was the latest stage of which material was available. It would be interesting to determine from older imagoes whether the œnocytes entirely degenerate and are taken up for the purpose of general nutrition of the tissues, thus affording a reserve food supply in addition to their secretory function. This latter view would, however, be hard to understand considering the short life of these insects, and also the fact that egg-laying goes on shortly after emergence from the pupal case.

Various functions have been attributed to the œnocytes. Landois (1865) gave to them the name "Respirationszellen" and assigned to them a place in the respiratory system. Graber (1873), in his early work, speaks of them as unicellular glands, the secretion of which was of unknown function; later, however, he holds that they are metamorphosed into the fat-body, and give rise to blood corpuscles. Berlese (1899) considers the œnocytes as excretory in function, as does also Koschevnikov (1900).

Anglas (1900) says: "Ce fait permet d'affirmer qu'ils sécrètent autour d'eux des ferments. Aussi les considérons-nous comme des cellules glandularies (nées de l'hypoderme) et jouant le rôle de glandes à sécrètion interne, mais de glandes dissociées." Verson (1891–2) considers them as secretory in nature as do also Rössig (1904) and Weissenberg (1907).

Holmgren (1900) in *Apion flavipes*, experimenting by injecting small quantities of powdered methylene blue and alizarin-cyanin into the body cavity, came to the conclusion that they were excretory in function. He found that the œnocytes took up the color in much the same manner as the Malpighian tubes, the nucleus of the œnocyte showing signs of staining somewhat earlier than the nuclei of the latter organs. Three hours after injection,

he found that the methylene blue had passed into the lumen of the Malpighian tubes in sufficient quantities to color them; the œnocytes at the end of an hour and a half had lost their color.

Following out this line of investigation, a dozen or more specimens of *Platyphylax designatus* were injected with an aqueous solution of methylene blue and the parts dissected and removed to a glycerine mount. It was found that immediately after injection the œnocytes and the spinning-glands, so prominent in this form, had both taken up the stain—the œnocytes to a much less marked degree than the latter. In only one instance, was it found that the Malpighian tubes had taken up the methylene blue, and in this case the color was seen in but a single tube. The œnocytes took up the stain more in the nucleus than in the cytoplasm, the latter, however, being distinctly tinged. The larvæ killed a half hour after injection showed the œnocytes more deeply stained, but no coloration was observed in the Malpighian tubes. Larvæ killed one hour after injection showed that the Malpighian tubes had begun to take up the blue color, but that the œnocytes and spinning-glands at this time were becoming less intense in their coloration. These results would lead one to rather different conclusions than those reached by Holmgren. It would be inferred from the similarity of the reaction of the œnocytes and spinning-glands as detailed above, that the former are secretory though of a different nature and intensity to that of the spinning-glands.

This view is further strengthened by injection experiments with sulphindigotate of sodium. Fürth (1903) says: "Die exkretorische Bedeutung der Malpighi'schen Schläuche offenbart sich auch in unzweideutiger Weise durch ihr Vermögen die Ausscheidung von Farbstoffen zu bewerkstelligen." Schindler (1878) found by injection of a solution of sulphindigotate of sodium into the body cavity of *Gryllotalpa* that the Malpighian tubes excreted the substance within twenty-four hours.

An aqueous solution of sulphindigotate of sodium was injected into the abdomen of a dozen or more larvæ of *Platyphylax designatus* 15–20 mm. in length, the insertion of the hypodermic syringe being ventral and thoracic, but the solution extending thoroughly

into all the space between the hypodermis and the digestive tract. Specimens killed within an hour after injection and mounted as in the former experiment, showed a slight accumulation of the solution in the lumen of the Malpighian tubes. This was even more marked two and a half hours and four hours after injection, a diffuse blue appearance being then very apparent throughout the cells of most of the tubes. The œnocytes, however, showed no signs of coloration at any of the periods observed nor any evidences of excreting the substance injected.

Just what the function of these peculiar cells may be it is difficult to say. In no case have I observed in the cytoplasm of any of the œnocytes what could be interpreted as accumulations of solid products of excretion. Their behavior toward methylene blue, and sulphindigotate of sodium would seem to show that they are secretory rather than excretory in function. The nature of their secretion is difficult and practically impossible to determine. Measurements show their greatest size in *Platyphylax designatus* to be in the pupal stage, and evidences from a purely structural basis seem to point towards the greatest functional activity during the late larval and the pupal stages. Their intimate relation to the fat-bodies seems vastly more significant than the occasional grouping about a tracheal tube. Their relative increase in size, while of course proportionate to the increase in size of the body, seems to bear a direct relation to the size and development of the fat-bodies. It is also found that their identity is retained throughout the period of metamorphosis. Can it be that their function is the secretion of a substance or enzyme which is of aid to the fat-body in its constructive work or is it that their function is, as Anglas (1900) suggests, that of internal glands of a dissociative nature, their secretion serving to aid in the elaboration of products for general nutrition of the tissues or for the disintegration of larval cells destined to disappear? These are very pertinent questions, but ones to which a definite answer cannot as yet be given. It seems safe to conclude that they are internal, ductless, secretory glands, whose function may, in the light of recent progress in the field of internal secretion, be for a long time a matter of conjecture.

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Explanation of Plate.

All figures drawn with a camera-lucida. × 630.

Fig. 1. Œnocyte from a 4.5 mm. larva.

Fig. 2. One of the smaller œnocytes from a 6 mm. larva.

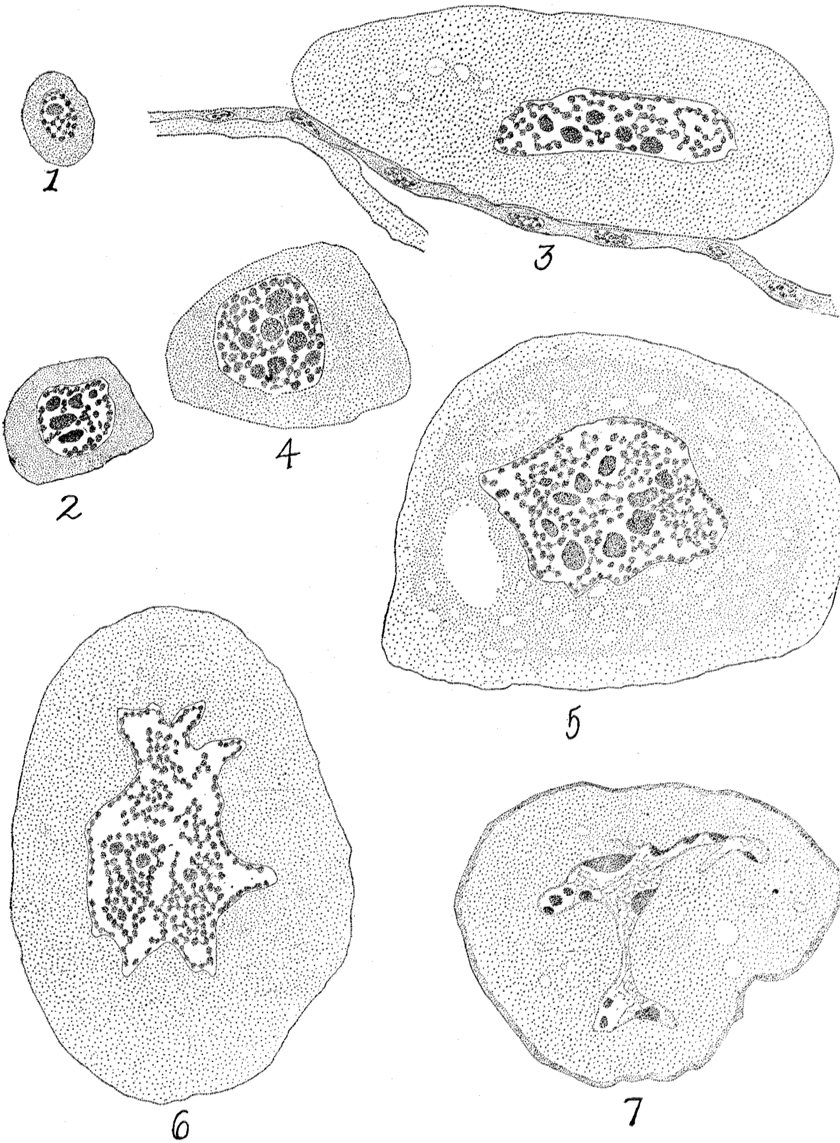
Fig. 3. One of the larger œnocytes from a 6 mm. larva.

Fig. 4. Œnocyte from a 12 mm. larva.

Fig. 5. Œnocyte of late larval period showing vacuoles in cytoplasm.

Fig. 6. Œnocyte of pupal stage.

Fig. 7. Œnocyte from newly emerged imago, showing signs of degeneration.



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